FILE COPY FOREST INSECT LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA.

463.1
~~465~~

UNITED STATES DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY

OFFICE OF FOREST PATHOLOGY

Title: INOCULATIONS OF PONDEROSA AND JEFFREY PINE SEEDLINGS WITH THE FUNGUS HORMISCIUM GELATINOSUM HEDGC., ASSOCIATED WITH A WEEVIL (CYLINDROCOPTURUS SP.) ATTACKING THESE HOSTS

Author: J. L. Mielke

JMM
MLL
KAS
JEP
GAS
HLM
ASW
JWB
~~ORE~~
RCH
JSY
STC
PCJ
ESS

To .....
From .....
For information
For criticism
To be published in.....
Please forward to .....

Date December 16, 1940, 193

U. S. GOVERNMENT PRINTING OFFICE: 1938 J07970

INOCULATIONS OF PONDEROSA AND JEFFREY PINE SEEDLINGS WITH THE FUNGUS HORMISCIUM GELATINOSUM HEDGC., ASSOCIATED WITH A WEEVIL (CYLINDROCOPTURUS SP.) ATTACKING THESE HOSTS

By
J. L. Mielke

Introduction

Preliminary investigations were made in 1939 by Mr. C. B. Eaton of the Bureau of Entomology and Plant Quarantine, Forest Insect Laboratory, of severe killing of 6 and 7 year old ponderosa and Jeffrey pine transplants in the Big Springs brushfield on the Lassen National Forest[1/]. Younger trees in the plantation had practically escaped injury. The individuals most commonly killed ranged from about 2 to 3 feet in height. Mortality was somewhat higher in ponderosa than Jeffrey pine. A limited amount of killing of reproduction was found in natural areas immediately adjacent to the brushfield and for many miles therefrom. The investigation also disclosed that a weevil (Cylindrocopturus sp.), of which little was previously known, was primarily responsible for the damage.

One of the characteristics of the infested material first noted by Mr. Eaton was the heavy blue-staining of the wood. According to Dr. Zentmyer[2/], formerly of this Division and who also made certain surveys of this damage, stain is not always present over the entire region covered by weevil borings; some of the wood in which weevils have been working may be entirely free from stain. Stain was always found near the lower limits of weevil infestation, however. Dead needles on entirely dead trees usually showed needle spots caused by feeding of weevils. In one case a young dead seedling was found to have abundant blue stain but no evidence of weevils. In a contrary instance, a young branch tip of a 1934 seedling was dead; on examination weevils were found to be present but there was no sign of blue stain. Otherwise the two organisms were universally associated in diseased seedlings.

1/ Eaton, C. B. Progress report on the biology of Cylindrocopturus longulus Lec. in ponderosa and Jeffrey pine reproduction. Bur. Ent. & Pl. Quar., Forest Insect Lab., Berkeley, Calif., pp. 1-18, illus. Feb. 1, 1940.

2/ Zentmyer, G. A. Jr. Memorandum on Big Springs Plantation. 3 pp. (Typed). Div. For. Pathology, San Francisco, Calif., June 27, 1939.

Several Jeffrey pine seedlings from the plantations were sent to Dr. C. T. Rumbold, Bureau of Plant Industry, Division of Forest Pathology, who reported3/ that the fungus Hormiscium gelatinosum developed in the majority of cases where cultures were made from wood, bark, larvae, pupae, and dead adults, as well as from feeding spots in the needles. The association of H. gelatinosum with all the active stages of the weevil indicated the possibility that this organism played an important part in bringing about the death of the tree. Also, it seemed probable that spread of the fungus from diseased to healthy trees might be dependent on activities of the insect since the organism was recoverable in a high percentage of cases from adults and from spots on the needles caused by adult feeding.

Hormiscium gelatinosum is known to be of economic importance in the staining of lumber. Its pathogenicity, however, has never been determined. Because of this fact and the severe damage that had occurred it was deemed advisable to determine if the fungus was capable of killing young trees independent of the insect. Accordingly, inoculation tests to provide evidence on this point were undertaken.

Inoculations in 1939

On September 6-8, 1939, Dr. G. A. Zentmyer inoculated 18 ponderosa and 22 Jeffrey pines. These were healthy-appearing, natural seedlings growing at three different localities in the open-mature, yellow-pine forest and immediately adjacent to the brushfield plantation. The trees ranged from about 1/2 to 4 feet in height and averaged about 2 feet. The inoculum used was spores of H. gelatinosum that developed in pure cultures of the fungus isolated and grown from affected material obtained in the plantation. In 17 of the trees the inoculations were made in the phloem and sapwood region with the aid of a corkborer, in 18 trees in the cortical region after making a small slit through the bark with a scalpel, and in 5 trees in the leaves after puncturing them with a needle. All cuts were covered with adhesive tape and most of the needle punctures were covered with a light coating of collodion. Examinations were made during the spring and summer of 1940. In no case was any evidence of the fungus observed in the checks or were lesions found to have resulted on any of the inoculated trees.

According to Eaton the adult weevils may start emerging in late May, are most numerous from about mid-June to mid-July, and few if any are to be found as late as the first week in August. Circumstances,

---

3/ Rumbold, C. T. Preliminary report on Jeffrey pine seedlings infested with the weevil Cylindrocopturus longulus Lec. Bur. Pl. Ind., Div. For. Pathology, Madison, Wisc., pp. 1-2, illus. May 24, 1939. (Typewritten).

however, did not permit making the inoculations until early September. At that late date in the season the trees were past their most active growth period. Also, both day and night temperatures were lower than during the summer and these were followed by winter temperatures possibly too low for growth of the fungus. Upon completion of the second examination, made June 22, 1940, and at which time no evidence of parasitism by the fungus was found, it was thought that the time of year the inoculations were made might possibly have been a factor in the negative results. Consequently, it was decided to make another set of inoculations in 1940 while the weevils were still in flight, the trees still highly active photosynthetically, and the temperatures relatively much higher.

Inoculations for 1940

The second series of inoculations were made July 11-13, 1940 by Mr. Eaton and the writer. In one lot a total of 65 healthy-appearing natural seedlings, 31 of them ponderosa pine and 34 Jeffrey pine ranging in height from 1 to 4 1/2 feet, with an average height of somewhat under 2 1/2 feet, were selected for the test. These were growing in a very open forest stand immediately adjacent to the three locations where Dr. Zentmyer made his inoculations and were judged by Mr. Eaton to be comparable to those which the weevils had been found to attack in nature and to be growing under comparable conditions.

In this test the methods employed in the inoculations differed somewhat in details from the previous ones. V-shaped, diagonal cuts and vertical slits were used. These were made through the bark and for a short distance into the wood since both are penetrated by the insect. Two incisions, at varying distances apart, were made per tree and the fungus introduced into both; or one was used as a control, in which case sterile medium was inserted. There was a total of 96 inoculations and 34 controls. In the case of vertical slits the bark was loosened for a short distance on one side and slightly raised with a scalpel so as to permit introduction of the fungus under it and next to the wood. Instead of spores, as previously used, an ample quantity of the fungus together with some of the medium on which it was growing was introduced into the incisions. The latter, which in all cases were made on stems, were at various heights between about 1 to 26 inches from the ground. Diameters on the stems at the point of inoculation averaged about .66 of an inch with a minimum of .3 inch and a maximum of 1.2 inches. All incisions were protected with a covering of adhesive tape. The trees themselves were in no manner protected from possible insect attack, this being considered as not worth while under the circumstances. No inoculations were made in needles.

The inoculum used was prepared by Dr. Rumbold. From weevil-infested Jeffrey pine seedlings obtained in the plantation and supplied her by Entomology and Plant Quarantine she isolated the fungus from a larva cut out of a gallery and from stained wood. Culturing of the fungus was on three different media, viz., cooked corn meal, rice, and wheat. Alternate

use was made of these in the inoculations and of sterile corn meal, rice, and wheat in the controls.

As a check on possible differences in virulence between isolates of Hormiscium gelatinosum from the weevil-infested trees and from other sources an additional lot of 10 ponderosa and 10 Jeffrey pine seedlings were also inoculated. These were similar in age and size to the trees mentioned above and all were growing at one of the localities used in the other test. The inoculum was also prepared by Dr. Rumbold. Two isolates of the fungus were used, one from window sash of sugar pine and the other from window sash of yellow pine. The inoculation procedure employed was the same.

An examination of all of the trees was made by Dr. W. W. Wagener and the writer on October 12. All were found to be healthy in appearance at that time except one that had been attacked by the weevils during the summer and died as a result. In the 85 trees, 21 of the inoculations showed a trace of stain in the wood but in no case did it extend in depth beyond the first annual ring. No lesions, however, were produced as a result of the inoculations with the different isolates of H. gelatinosum used. No evidence of any fungus was apparent in any of the controls. The results were considered negative.

Results and Conclusions

Inoculations with Hormiscium gelatinosum made in 1939 and 1940 in healthy, natural seedlings of ponderosa and Jeffrey pine growing near Big Springs on the Lassen National Forest all resulted negatively. Consequently it is concluded that the various isolates of this fungus used in the tests are not pathogenic. In the case of seedlings of these pines that have been attacked by the weevil (Cylindrocopturus sp.), and in which the fungus may then be found, it appears that the latter is of but secondary importance contributing to the dying, if at all, only by a possible hastening of the process after its initiation by the weevils.